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Characterization by Fluorescence and Electron Microscopy In Situ Hybridization of a Double Y Isochromosome

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A patient with mixed gonadal dysgenesis and Y isochromosomes i(Y) is described. Lymphocyte cultures from peripheral blood contained a high proportion of 45,X cells and several other cell lines with two different marker chromosomes (mars). These markers had either a monocentric (mar1) or a dicentric appearance (mar2). Following high-resolution GTG, RBG, QFQ, and CBG bandings, five cell lines were identified; 45,X/46,X,+mar1/46,X,+mar2/47,X,+mar1x2/47,X,+mar2x2. The percentages were 66/6/26/1/1%, respectively. Chromosome banding analyses were insufficient for characterization of the markers. In situ hybridization of specific probes for the Y centromere and its short arm showed, both in fluorescence and electron microscopy (EM), two different Y rearrangements. Mar1 is an isochromosome for the short arm i(Yp) and mar2 is a dicentric which was shown by EM to be a double isochromosome Yp, inv dup i(Yp). The breakpoint producing mar1 is within the centromere and the one producing mar2 is within one of the short arms of the Y isochromosome. The findings of different cell populations in peripheral blood lymphocytes indicate the postzygotic instability of this i(Yp). © 1996 Wiley-Liss, Inc.

KEY WORDS: double Y isochromosome, fluorescence and electron microscopy in situ hybridization

INTRODUCTION

In prenatal studies, marker chromosomes (mar: unidentified abnormal chromosome) are found in about 1.5 per 1,000 fetuses [Sachs et al., 1987] and in about 1.2 per 1,000 newborns [Buckton et al., 1985]. The characterization of the marker is important; it allows phenotype-genotype correlations. Genetic counselling and characterization of cases with marker chromosomes are still problematic for clinical geneticists. When the marker chromosome is not familial, the risk of phenotypic abnormalities is often unclear. Because of the complexity of many rearrangements of the Y chromosome and the constant presence of a 45,X cell line, the clinical spectrum of patients with 46,X,+mar karyotypes ranges from normal male phenotype to normal female phenotype and includes oligospermia, hypospadias, ambiguous external genitalia, streak gonads, uterus, and tubes. In patients with de novo 46,X,+mar karyotypes, ascertained because of genital anomalies or fertility problems, it is crucial to know whether the markers are of Y origin because gonadoblastoma may develop in the presence of a Y chromosome in a female phenotype [Krasna et al., 1992]. Unfortunately, it is often impossible to determine by standard cytogenetic techniques, whether the aberrant chromosome is derived from a Y or an X chromosome and whether a segment of the Yp is deleted or not. Because of the low specificity of the banding pattern, it is difficult to differentiate, by cytogenetic methods alone, an i(Yp) from a deleted Yq. Isochromosomes derived from a del(Yq) or del(Yp) are in general found in mosaicism with a 45,X cell line. Secondary rearrangements of the dicentric chromosome are possible, but often the derived chromosome cannot be identified.

We report here the case of a patient with mixed gonadal dysgenesis and a de novo 45,X/46,X,+mar1/46,X,+mar2/47,X,+mar1x2/47,X,+mar2x2 constitution. Mar1 was the size of a group G chromosome, and mar2 was the size of a group E chromosome. We used molecular cytogenetics and electron microscopy (EM) to characterize the marker chromosomes.

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MATERIALS AND METHODS

Clinical Report

The patient was referred for karyotypic analysis at the age of 19 years, because of primary hypogonadism. She presented a female phenotype with a mild growth delay, high arched palate, cubitus valgus, many pigmented naevi, and no breast development. Echography showed the absence of ovaries and the presence of a small uterus. Histologic examination demonstrated streak gonads (no follicles nor germ cells). The family history was not informative.

DNA Probes

The DNA probe pXBR1 is a chromosome X-specific DNA α satellite [Yang et al., 1982]. The pYα1 is a 6 kb Y alphoid unit; 91H4.5 is a 4.5 kb Hind III fragment cloned in pTZ18R of the DYZ4/DYZ5 repeat locus, located in the mid region of the Y short arm [Tyler-Smith et al., 1988]. It is the same sequence as GMGY10 [Affara et al., 1986]. The probes were labeled by nick-translation with biotin-11-dUTP (BioNick) according to the Gibco BRL protocol.

Chromosome Preparation and FISH Protocol

Peripheral blood lymphocytes were cultured in RPMI 1640 and harvested after cell synchronisation with thymidine. After fixation, the chromosome preparations were Q-, R-, G- and C-banded (QFQ, RBG, GTG, and CBG). In situ hybridization and detection were performed as described [Fetni et al., 1991, 1992; Lemieux et al., 1992] with minor modifications. Probe concentrations on the slides were 1 ng/μl for PYα1 and 3 ng/μl for 91H4.5. Detection was made with an anti-biotin antibody (Enzo Diagnostics, Inc.) and a biotinylated goat anti-rabbit IgG antibody (BRL). For gold labeling, this step was followed by an anti-biotin antibody and a protein A gold complex (3 nm gold particles). Selected mitoses were transferred from the slides to EM grids [Messier et al., 1986]. For fluorescence in situ hybridization (FISH), preparations were incubated with a streptavidin fluorescein isothiocyanate conjugate. After counterstaining with propidium iodide, the slides were mounted in antifading solution.

RESULTS

Analysis of 100 metaphases showed 66% of 45,X, 6% of 46,X,+mar1, 26% of 46,X,+mar2, 1% of 47,X,+mar1x2 and 1% of 47,X,+mar2x2 (Table I). FISH with a specific X-alphoid-satellite probe showed, in all observed metaphases and nuclei, only one signal in all the cells. Lymphocyte cells from a female with two normal X chromosomes served as control. Thus, the hypothesis of an altered X chromosome was ruled out.

CBG analysis of the marker chromosomes revealed one centromere for mar1 and two for mar2. QFQ analysis revealed no distal heterochromatic bands (data not shown). Banded metaphases did not reveal the characteristic distal fluorescent band of Yq.

Mar1 exhibited only one spot with the Y centromere probe pYα1 and appeared to be monocentric. Hybridization of 91H4.5, which detects repeated sequences at mid Yp, indicated that mar1 was indeed an i(Yp) by showing spots on both sides of the centromere (Fig. 1d,e) with the formula i(Y)(pter→cen→pter) (Fig. 3).

The R- and G-banding pattern of mar2 suggested that it might be an isodicentric Y chromosome with two copies of Yp and proximal Yq but without copy of distal Yq (Fig. 1a1,b1). Hybridization of pYα1, which is specific for the Y centromere, showed one signal for mar1 and two separate spots for mar2 (Figs. 1c,c1,c2, 2a), thus confirming the presence of two Y centromeres (in mar2), one of which appeared without the usual centromeric constriction, and was presumably inactive as would be expected for a dicentric Y chromosome. Hybridization of 91H4.5 on mar2 showed spots distal to each of the two centromeres. The nature of mar2 was only revealed after EM studies. Indeed after electron microscopy in situ hybridization (EMISH) of mar2 (contrary to what had been observed with FISH), three spots were visible indicating the presence of three sets of the proximal region of the short arms (Fig. 2b). Two large spots at the ends of the marker and one smaller spot exactly in the middle suggested a double i(Yp) structure for which we are suggesting the formula inv dup i(Y)(pter→cen→p11.2::p11.2→cen→pter). As shown in Table I, the final interpretation of the karyotype as based on R-, G-, C-, and Q-bands in combination with FISH and EMISH was 45,X/46,X,+i(Y)(pter→cen→pter)/46,X,+inv dup i(Y)(pter→cen→p11.2::p11.2→cen→pter)/47,X,+i(Y)(pter→cen→pter)x2/47,X,+inv dup i(Y)(pter→cen→p11.2::p11.2→cen→pter)x2. Mar2 is thus interpreted as being a double isochromosome joined at locus 91H4.5.

DISCUSSION

Many attempts have been made to characterize marker chromosomes cytogenetically, dividing them into different groups according to size and staining reactions. The presence of nucleolus-organizing regions (NORs) confirms the origin from the short arms of acrocentric chromosomes [Callen et al., 1992]. G-11 and C-bandings have been used to distinguish the X from the Y chromosomes [Magenis and Donlon, 1982]. Some have used DA/DAPI to identify nonfluorescent Y chro-

TABLE I. Percentage of Anomalies and Chromosomic Formulas of the Five Cell Lines Found in Lymphocyte Cultures

	%	Anomalies	Chromosomic formulas
X	66	−Y	45,X
mar1	6	i(Yp)	46,X,+i(Y)(pter→cen→pter)
mar2	26	inv dup i(Yp)	46,X,+inv dup i(Y)(pter→cen→p11.2::p11.2→cen→pter)
2 mar1	1	i(Yp)x2	47,X,+i(Y)(pter→cen→pter)x2
2 mar2	1	inv dup i(Yp)x2	47,X,+inv dup i(Y)(pter→cen→p11.2::p11.2→cen→pter)x2

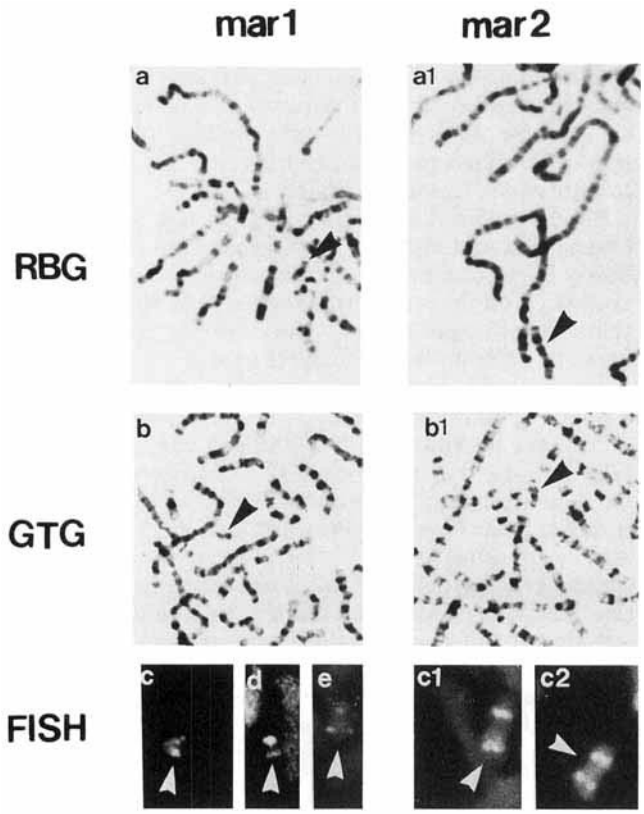

Fig. 1. Partial 46,X,+mar metaphases, presenting the tiny monocentric Y chromosome (mar1) and the dicentric (mar2) (arrowheads indicate mars in all cells). **a** and **a1:** RBG-banding. **b** and **b1:** GTG banding. **c, c1,** and **c2:** Markers hybridized with pYα1. **d** and **e:** Marker 1 hybridized with 91H4.5.

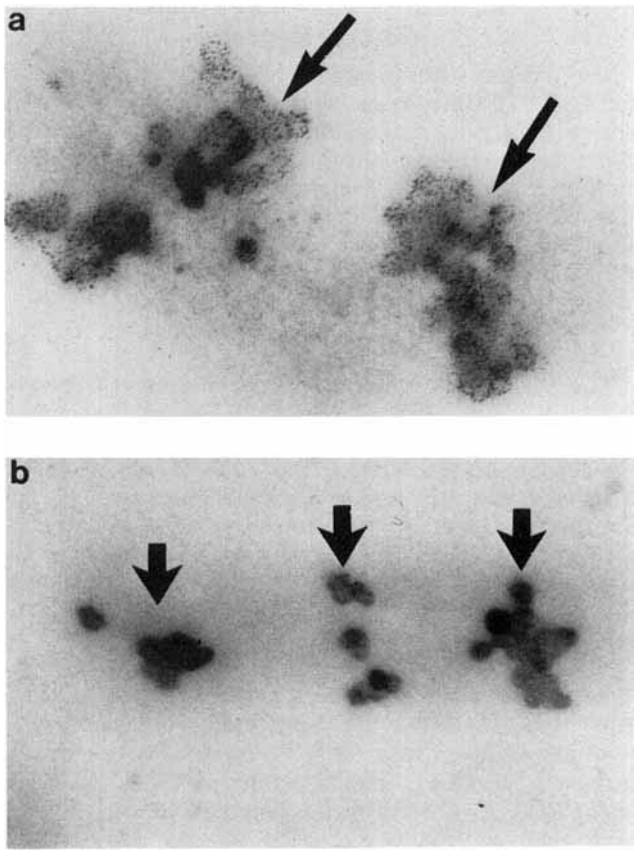

Fig. 2. Electron micrographs of mar2. The arrows point to EMISH signals. **a:** Hybridization with pYα1 shows two spots revealing two centromeres (~×15,000). **b:** Hybridization of 91H4.5 shows three sets of the DYZ4/DYZ5 repeat locus. The addition of one (median, smaller) is situated at the breakpoint (~×16,000).

mosomes in 45,X/46,XYnf mosaicism [Wisniewski and Hirschhorn, 1982], but the specificity of this staining has been questioned [Lin et al., 1990]. None of these methods has proved reliable enough to provide clearcut results to be used in genetic counselling [Gemmill et al., 1987].

Recently, many marker chromosomes have been characterized, especially when repetitive centromere-specific probes have been used [Rauch et al., 1992; Qu et al., 1992]. This has resulted, in some cases, in the delineation of syndromes such as the isochromosome 18p syndrome [Callen et al., 1990; Blennow and Bröndum-Nielsen, 1991] and the Pallister-Killian syndrome [Schinzel, 1991]. Nearly half of Ullrich-Turner syndromes are mosaics [Fryns et al., 1983]; in 5 to 6% of these cases, at least one cell line contains a normal or a modified Y chromosome. The case presented here belongs to the last group, but its originality derives from the very unusual type of Y anomaly found. The mosaic contains five cell lines, and our analysis of the lymphocytes revealed the relative proportions in blood (Table I). In mosaics, the effect of a Y chromosome is unpredictable. However, the presence of two different anomalies of the Y in an Ullrich-Turner mosaic presenting a characteristic phenotype may provide information on the localization and function of the genes concerned. One can already note that the presence of a modified Y cell line diminishes the shortening effect on stature, which is an important component of the Ullrich-Turner syndrome. Indeed the most important genes for Ullrich-Turner syndrome have been cloned and localized on Yp and Xq [Fisher et al., 1990].

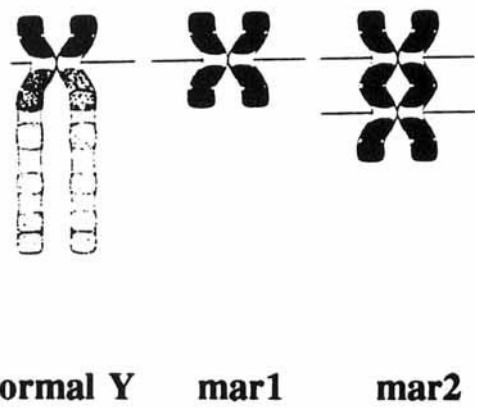

Fig. 3. Diagram illustrating the postulated Y rearrangement interpreted as an i(Yp),(mar1) and as an inverted duplication of i(Yp),(mar2).

In the case presented here, two markers are observed, the long one (mar2) deriving from the short one (mar1) and, as shown by EM, containing four copies of the proximal region of Yp. This had never previously been reported. We can speculate that the first event took place when a break in the centromeric region was followed by the production of an i(Yp) mar1. The third spot observed in EM in the middle region of mar2 and its constantly smaller size than that of the two distal spots suggest that the second event resulted from a break within the mid region of the Yp of one of the sets of short arms of the i(Yp), in the repeat locus which hybridizes with probe 91H4.5 (Yp11) [Tyler-Smith et al., 1988]. An inverted duplication following this event produced the second marker (mar2). The two other cell lines resulted from non-disjunctions. The initial cell line must have contained a Y, possibly an abnormal one. The female phenotype associated with the isodicentric Yp is presumably a consequence of the mosaicism of the marker [Daniel, 1985]; a large proportion of cells in critical tissues probably having lost the abnormal Y chromosome. Unfortunately, the cytogenetic studies of the removed gonads could not be done.

We clearly see here the impact of combining in situ hybridization and electron microscopy to precisely characterize unusual markers. The identification of the marker as a modified Y is of great preventive importance, since 10 to 15% of patients with abnormal Y chromosomes and dysgenetic gonads are at risk of developing gonadic neoplasias [Verp and Simpson, 1987]. Thus, in the cases with a Y chromosome, the gonads are removed. However, the marker chromosome often cannot be determined without ambiguity by classical cytogenetic methods. Using molecular cytogenetic techniques and electron microscopy, it is possible to characterize any marker chromosome and to avoid surgical procedures when only X chromosome material is present.

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